molecules did not accumulate in clathrin-coated pits that were not associated with GLUT4 domains.
Based on these data we propose fence-based model for retention and accumulation of GLUT4 at specific domains of the plasma membrane responsible for active delivery and internalization of GLUT4 distinctly from other proteins undergoing constitutive recycling.

**1629-Pos Board B399**
**Microtubule-Driven Migration of Clathrin-Coated Pits Towards Vesicle Fusion Sites for Rapid Recycling in Pancreatic Beta Cells**
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Due to the long time required for de novo formation of deeply invaginated clathrin-coated pits that mostly appear at sites different from exocytosis before fission, clathrin-dependent vesicle recycling in secretory cells is usually regarded as a slow process. Here we show that regulated exocytosis in pancreatic beta cells is associated with glucose-dependent recruitment of clathrin and dynamin-1 puncta at the release sites shortly or long after fusion pore opens. The subsequent disassembly of these clathrin puncta mediates internalization of synaptotagmin VII clusters in the plasma membrane. The fast clathrin-dependent endocytosis contributes significantly to the total vesicle recycling process, and involves microtubule-dependent linear movement of preformed clathrin-coated pits to vesicle release sites prior to fission. These results have revealed an unexpected close spatio-temporal coupling of clathrin-dependent endocytosis to vesicle fusion in pancreatic beta cells, and highlighted a novel pathway to replenish the ''readily retrieval pool'' of clathrin that sustains fast clathrin-dependent endocytosis under intense stimulation.

**1630-Pos Board B400**
**Clathrin Self-Assembly into Polyhedral Cages Studied by Computer Simulations**
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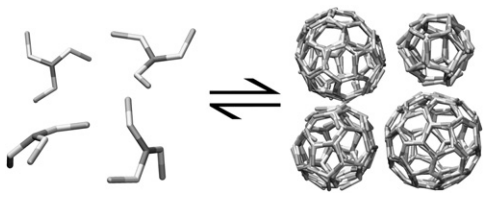
Clathrin is a three-legged proteins that self-assembles into polyhedral cages with regulatory and mechanical functions in the formation of cargo-laden vesicles at the cell-membrane and trans Golgi network. The essential features of self-assembly are innate to clathrin, as cages are also formed in purified slightly acid solutions. Our simulations of this process using a highly coarse-grained clathrin model reveal that a non-uniform distribution of interactions over clathrin's surface, rather than its characteristic shape, holds the key to self-assembly [1]. The cages are polydisperse, with a strong preference for a small subset of all possible configurations with twelve pentagonal and a variable number of hexagonal faces. Based on the experimental critical assembly concentration, we deduce an average binding energy of ~23kT per clathrin [2]. The simulations also answer the long-standing question of how a flat purely hexagonal clathrin lattice can produce a cage with twelve pentagons: the introduction of spontaneous curvature through a change of hub and/or knee puckers causes tensions that result in the release of dome-shaped fragments, which may subsequently grow into full cages by recruiting cytosolic clathrin [2].



[1] Biophys. J. 99, 1231 (2010).
[2] Traffic 12, 1407 (2011).

**1631-Pos Board B401**
**Stochastic Nature of Clathrin-Coated Pit Assembly**
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The dynamics of clathrin coated pit (CCP) formation, observed through total internal reflection fluorescence microscopy, shows considerable diversity. Foremost is fate divergence, which leads to ''abortive'' and ''productive'' pits, i.e., structures which, respectively, do or do not mature into clathrin coated vesicles (CCVs). Also, there is notable heterogeneity in the lifetimes of abortive pits and the apparent time to the completion of productive CCPs. We explore the extent to which the stochastic nature of CCP growth can explain these observations. For this purpose we analyze a simple model that includes a kinetic scheme for CCP assembly and a related functional form for free energy of CCP formation. Using this model, we calculate the lifetime distribution of abortive pits (via Monte Carlo simulation) and fit it to experimental data to determine the exact effective potential experienced by CCPs. We show that the CCPs without cargo are energetically unstable, and that the binding of cargo might stabilize a CCP and thereby facilitate CCV formation. Finally, we estimate how variation in the time of CCV formation is linked to the stochastic associations and dissociations of coat components.

**1632-Pos Board B402**
**Dynamin I Regulates Activity-Dependent Fusion Pore Dilation via a Calcineurin-Dependent Pathway in Mouse Adrenal Chromaffin Cells**
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Chromaffin cells of adrenal medulla utilize two modes of exocytosis in order to achieve a proper response to sympathetic input under basal tone as well as the sympathetic stress response. Under sympathetic tone, modest synaptic excitation drives chromaffin cells to selectively secrete modest levels of catecholamine through a restricted fusion pore. In contrast, elevated sympathetic activity experienced under acute stress results in dilation of fusion pore to achieve maximal catecholamine release and to facilitate release of co-packaged peptide transmitters. Therefore, the dilation of fusion pore is the key control point for the activation of the sympatho-adrenal stress response. Despite the physiological importance of this process, the molecular mechanism for how it is achieved is still unclear. Here, we employ electrophysiological, electrochemical, and fluorescence based approaches to investigate hypothesized signaling pathway for the regulation of activity-mediated fusion pore expansion. We show that dynamin I is dephosphorylated by calcineurin only under high stimulation. Calcineurin-mediated dephosphorylation of dynamin I leads to the recruitment of syndapin-N-WASP. Disruption of each step of this cascade results in limited fusion pore dilation. Our results suggest that fusion pore dilation is regulated by a calcineurin-dependent dephosphorylation of dynamin I.

**1633-Pos Board B403**
**Structural Analysis of Dynamin Reveals Power Stroke**
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D Dynamin family members are large GTPases involved in membrane fission and fusion events throughout the cell. The founding member, dynamin, plays a major role in vesiculation events during endocytosis, synaptic membrane recycling, and membrane trafficking. The current model predicts dynamin wraps around the necks of coated pits and upon GTP hydrolysis dynamin constricts and disassociates from the lipid, which then leads to membrane fission. In support of this model, purified dynamin self-assembles into spirals around lipid, generating dynamin-lipid tubes that constrict, twist and fall off upon GTP addition. To determine the conformational changes that occur during GTP hydrolysis, we calculated 3D maps of dynamin by cryo-electron microscopy methods. Here we present our latest 3D map of ΔPRD-dynamin (resolution of 12 Å) with three crystal structures docked into our map, the GMP-PCP GG domain (GTPase domain-GED fragment), the stalk domain from another dynamin family member, MxA, and the PH domain from dynamin. Based on the docking results, we predict the location and interactions between the domains. In addition, comparison between the GTP-bound state (GMP-PCP) and transition state (GDP.AlF4-) within the GG construct suggests that the conformational change induced by GTP hydrolysis drives a large swing of the BSE (bundle signaling element). We predict that the BSE movement is dynamin's power stroke that results in a significant twist and constriction of the underlying lipid bilayer leading to membrane fission. Recently, we have calculated a 3D map of full-length dynamin in a further constricted state, with a resolution of ~15Å. The inner lumenal diameter of this structure is ~2-4 nm, a range that is compatible with spontaneous lipid fusion. Currently we are docking the crystal structures into our K44A-dyn map to identify changes within dynamin domains that leads to maximum constriction and ultimately membrane fission.